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DIAGNOSIS OF PORCINE RESPIRATORY DISEASE WITH A REAL-TIME PCR DIAGNOSTIC PACKAGE

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Real-time PCR, swine, respiratory disease, diagnostic package

Introduction

Pigs often show symptoms of respiratory disease. For optimized diagnosis, a diagnostic package was developed at the National Veterinary Institute (NVI) in Copenhagen, Denmark. With this package, lung tissue samples from 3 pigs are simultaneously tested for swine influenza virus (SIV), porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumonia* (Mhyo). Test for porcine reproductive and respiratory syndrome virus (PRRSV) is an optional part of the package. The infections included in the package, were selected from their differential diagnostic significance and because they cannot be distinguished by macroscopic evaluation alone.

The package is offered with a shorter turn-around time and a lower price compared to individual testing, reasoned by the higher through-put analysis made possible, when the total number of samples submitted to the laboratory elevates. The swine respiratory diagnostic package was launced at the end of 2007 and is presently available as a diagnostic service at NVI.

Here we evaluate the results from samples submitted for routine diagnostics with the package until 1st quarter 2010, and the impact of offering the package on swine respiratory disease diagnosis of NVI submissions.

Materials & methods

Lung tissue samples were submitted to the NVI from veterinary practitioners as 3x3x3 cm pieces of lung tissue from 3 individual pigs with respiratory disease from the same pig herd. RNA was extracted with RNeasy Mini Kit (QIAGEN) and DNA was extracted with QIAamp DNA Mini Kit (QIAGEN). RNA was tested for the presence of SIV and optionally for PRRSV, and DNA was tested for Mhyo and PCV2. Testing was performed with separate real-time PCR assays for SIV, PRRSV, Mhyo and PCV2, respectively. SIV and Mhyo results were reported as detected/not detected. PRRSV results were reported as EU or US type detected/not detected. PCV2 results were quantitative, expressed as copies of PCV2 pr. 500 ng DNA extracted.

Results

The number of submissions for the swine respiratory disease diagnostic package gradually increased during 2008. PRRSV test was opted for in only few cases (table 1). SIV was the pathogen found most often, followed by Mhyo and PCV2 $>10^7$ copies.

SIV and PRRSV real-time PCR tests are also offered from NVI as single tests, whereas Mhyo and PCV2 real-time PCR analysis of lung tissue generally is only conducted as part of the package. The number of SIV submissions to NVI which were not part of the package, was 65 in 2008, 101 in 2009 and 6 in 1st quarter of 2010. For comparison, before launch of the package, there were a total of approximately 100 submissions for SIV analysis per year of which around 20 % were positive. With implementation of the diagnostic package, the number of samples pr. submission has increased to an average of 2.5 samples pr. submission in 2010. PRRSV analysis was requested mainly as part of the package, however this analysis was opted for in only very few cases.

Table 1: Number of submissions for swine respiratory disease diagnostic package from launch 4th quarter 2007 to 1st quarter 2010, and share of submissions with at least one positive sample or with $>10^7$ copies of PCV2 pr 500 ng DNA.

	Diagnostic package Number of submissions and findings (%)				
	total	SIV pos.	Mhyo pos.	PCV2 $>10^7$	PRRSV Opted
2007/4	31	8(26)	6(19)	4(13)	6
2008	224	57(26)	52(23)	33(15)	9
2009	198	55(28)	46(23)	28(14)	11
2010/1	37	16(43)	8(22)	4(11)	14

Discussion & conclusions

The total number of submissions to NVI for diagnosis of respiratory disease in pigs increased with launch of the diagnostic package, as measured from submissions for SIV analysis for the past 5 years. The reason for this is most probably due to the offered shortened turn-around time of samples, in combination with reduced price and increased marketing of the diagnostic potential.

SIV was the pathogen most often detected, indicating swine influenza as a major cause of respiratory disease in Danish pig herds. The proportion of SIV positive submissions increased simultaneously with launch of the diagnostic package, from around 20 % positives before till 26-28 % positives after launch. This may reflect a more safe diagnosis at the herd level due to a higher number of samples per submission/herd. Although this is only showed for SIV diagnosis, a similar effect is inferred for the diagnosis of other infections included in the package, compared to single sample submissions, which earlier on was often chosen by the customers in order to cut down the expenses to laboratory diagnostics.

The proportion of positives for all infections has remained stable since launch of the package, but with a marked rise for SIV in 2010. The number of submitted samples in 2010 is however low, so this increase may not reflect a true rice in the proportion of SIV positive Danish pig herds. The reason for fewer submissions in 2010 is most probably related to the outbreak of Pandemic Influenza virus A H1N1(2009) in the human and swine populations worldwide, since detection of this virus in Danish pig herds would impose restrictions on movement of pigs to the herd. This ban was, however, lifted in May 2010.

The implementation of this diagnostic package offering test of more samples per submission for a relatively low price has resulted in safer diagnosis at the herd level and in more submissions for diagnosis, showing that diagnostic packages may contribute to improved diagnostics.